NERVE AGENT ANTIDOTES IN THE 90's:

A CHANGE IN PARADIGM

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I. Introduction:

Nerve agent (organophosphate) detection, symptom identification, and treatment has long been a priority for US military forces, agent storage depots, demil facilities, and now is becoming an issue at formerly used defense sites (FUDS) or in the case of Rocky Mountain Arsenal (RMA), a former production facility, where these agents had been stored, tested, or disposed of and which is currently undergoing environmental remediation.

Protecting workers who may be potentially exposed to acute or chronic releases of nerve agents during demilitarization or environmental clean up operations are one of my many responsibilities as the Safety Manager/ Industrial Hygienist for RMA.

FUDS and inactive installations like RMA pose a unique set of problems when chemical munitions or agent contaminated piping or vessels are encountered. RMA, like other inactive installations or FUD sites undergoing environmental restoration, does not have an active chemical mission and lack the supporting infrastructure for emergency response, trained back up chemical workers, onsite medical staff, etc. which most storage depots and chemical demilitarization facilities have available to respond to a chemical event. RMA does have an advantage over most FUD sites and other locations where chemicals and in particular, suspect nerve agent munitions may be found. Our facility has a fully instrumented and staffed Environmental Laboratory who have an extensive background in agent chemistry and we have two operational Real Time Analytical Platforms (RTAP's) which can respond to sample suspect agent found during remediation.

The lack of these onsite resources makes it imperative that we, the risk managers, be able to find and evaluate, not only new detection and protective devices, but also new and improved methods of treating potential chemical agent casualties, especially nerve agent casualties at future FUD sites and other locations like RMA.

II. History of Nerve Agents:

The discovery of nerve agents are now very well documented but were a mystery to Allied forces during World War II. Dr. Gerhard Schrader was working on finding a new

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organophosphate pesticide for IG Farben, a german manufacturing giant, when in 1937, his personel accidently spilled a small amount of the pesticide, Tabun on a lab bench.

His assistants suffered from contracted pupils, dizziness, and had difficulty breathing. These symptoms persisted for several weeks and their work on this pesticide was immediately transferred to the german army chemical laboratory complex located near Berlin-Spandau. This was the beginning of nerve agent research and production.[1]

Treatment for nerve agent poisoning has not progressed much beyond what was available in the early days of nerve agent and organophosphate pesticide production.

The standard treatment protocol involves administration of atropine and an oxime to relieve the effects of the agent and reactivate the target enzyme, acetylcholinesterase (AChE), respectively. Recent research has indicated that several pretreatment regimens may offer better results by reversibly binding with the target enzyme or acting as a "scavenger" of the circulating nerve agent.

The use of a scavenger or a similar enzyme may actually prevent the occurrence of severe post-exposure incapacitation such as convulsions or behavioral impairments associated with nerve agent intoxication, and may work by itself, eliminating the need for atropine and oxime as a postexposure treatment.

In my presentation, I will compare and contrast these different treatment approaches and offer recommendations on which approach may be most beneficial for work at a remote clean up site.

III. Chemical Structure and Physiological Action:

Nerve agents are a member of the organophosphate pesticide family. There are four nerve agents which are commonly encountered; ethyl N, N-dimethyl phosphoramidocyanidate, ((Tabun or (GA)), isopropyl methyl phosphoroflouridate ((Sarin or (GB)), pinacolyl methyl phosphonoflouridate ((Soman (GD)), and ethyl S-2-diisopropyl aminoethyl methylphosphorothiolate (VX). All four agents has a phosphorus atom double bonded to an oxygen as the central site of activity.[1]

I will restrict my presentation to Sarin and Soman because they are the most toxic (Sarin having the lowest median lethal dose ((70 mg-min/m3)) and Soman because it can rapidly "age" which complicates reactivation of its target enzyme, Acetylcholinesterase.

Nerve agents act by the irreversible binding and inhibition of the enzyme acetylcholinesterase (AChE), the enzyme normally responsible for hydrolysis of acetylcholine (ACh) located at neural synapses. This binding leads to accumulation of ACh at post synaptic cholinergic receptors throughout the body. Symptoms of "classic" nerve agent poisoning (cholinergic stimulation due to accumulation of acetylcholine) are presented in Table 1.[2]

Muscarinic and nicotinic receptors (or effects) are those receptors in both the parasympathetic and sympathetic nervous systems which respond to either muscarine or nicotine, respectively, and these are signs those receptors exhibit when stimulated.

TABLE 1

SIGNS OF EXCESSIVE CHOLINERGIC STIMULATION

Muscarinic:	Increased bronchial, salivary, ocular, and intestinal secretions; sweating, miosis, broncospasms; intestinal hypermobility; and bradycardia.
Nicotinic:	Muscle twitching; fasciculations; weakness; and paralysis.
Central Nervous System (CNS)	Loss of consciousness; convulsions; and respiratory depression.

CNS effects are due to the ability of nerve agents to cross the blood-brain barrier and are of great concern because neither oxime or other pretreatments like Pyridostigmine Bromide can cross the barrier to prevent the trauma caused by nerve agents, in particular, Soman.

Conventional medical treatment for nerve agent poisoning includes a regimen of anticholinergic drugs (atropine) to counteract the accumulation of acetylcholine and oxime (2-PAM Chloride) used to reactivate agent inhibited AChE. Reactivation of inhibited AChE by oxime can generate enough active AChE to restore proper breathing (diaphragm and associated muscles) if the worker or soldier recognizes and reacts to the symptoms, the acquired dose (dermal, inhaled, or absorbed) was not excessive, and the treatment regimen of atropine and oxime are administered promptly.[6] However, these nerve agents can undergo a process called "aging", where the central phosphorus moiety of the agent can change which results in the inability of oxime to release or reactivate the sequestered AChE. GD has the fastest half-time or time required to age 50% of its agent-Ache complex. It's half-time is two (2) minutes, which underlines the need for rapid identification of symptoms and treatment or an alternative approach which does not depend on identification and treatment of symptoms.[6]

IV. Changing Our Paradigm Toward Nerve Agent Protection:

The use of atropine and oxime for post exposure treatment has several drawbacks:

First, we are still **REACTING** to symptoms and not removing (or treating) the cause, nerve agent poisoning. The individual suffering from the effects mentioned in Table 1 does not fully recover from those symptoms for several days and the treatments do not alleviate convulsions and other CNS effects associated with agent poisoning. The individual will be severely restricted in performing demil or hazardous waste work for several weeks following the exposure and the worker will have lowered levels of AChE and possibly diminished learning and cognitive skills for several months or longer.

Another drawback would be the availability of qualified medical personnel to administer antidotes and treatments not normally carried by the workers. The military has taught agent first aid where the individual or his "buddy" administers atropine and oxime intramuscular (i.m.) for both minor and advanced agent symptoms, respectively. The practice of self administration and buddy aid are also taught for workers at remote hazardous waste sites and chemical demilitarization facilities. Self and buddy aid does not address the treatment of convulsions and other symptoms which are not alleviated by atropine or oxime.

Treatment for convulsions include the use of Diazepam or Benactyzine which are administered by medical personnel who may not be present at remote operations or if additional antidotes are required to treat advanced symptoms since Army guidelines only allows three sets of the Mark I Nerve Agent Antidote Kit to be administered without competent medical authorities present.

Fixed demil and storage sites also have trained medical staffs which would augment and oversee additional supportive care while the remote sites and field personnel rely solely on self and "buddy" aid.

The final drawback to the current treatment protocol deals with the maximum benefit post treatment with atropine and oxime can provide. Studies have shown that the maximum Protective Factor (PF) for GD, which is the factor or ratio a treatment or pretreatment raises the lethal dose (LD) of a toxic agent is approximately 1.6, while a soldier or hazardous waste worker could possibly expect to absorb up to 5X the lethal dose during a catastrophic event such as a detonation of a munition nearby.[6]

Several scenarios exist where other than typical post exposure treatment of nerve agent poisoning would warrant the use of a pretreatment.

Examples include the Army Technical Escort Unit or a similar Explosive Ordnance Disposal unit responding to a leaking chemical munition at a FUD site or a Special Forces Team on a mission to destroy a suspected enemy agent facility (i.e. baby food production plant). These units must have the capability of administering treatments which if exposed, would provide adequate protection without relying upon extensive external medical support.

Fixed demil and storage facilities would also have a need for pretreatment when responding to a catastrophic event related to a explosion or mishap at the facility.

V. Pretreatment Alternatives:

1. CARBAMATES

A. Pyridostigmine Bromide (PB)

Pyridostigmine Bromide is a quaternary carbamate which does not cross the blood-brain

barrier and has been used to treat Myastenia Gravis but in much higher dosages (180-900 mg/day) than prescribed for pretreatment of nerve agent poisoning).[2]

PB competitively inhibits nerve agents by forming a reversible bond with AChE, which will dissociate with time releasing or reactivating the AChE. The prescribed dosage is 30 mg/ 8 hrs (three times daily) for a period not to exceed two weeks. The target level of sequestered plasma AChE is 30% +/- 10%.

PB does not offer any protection against nerve agents by itself, but when coupled with the posttreatment regimen of atropine and oxime, PB can effectively raise the protective factor (in non human primates) by a factor of at least five.[4]

Side effects from PB pretreatment especially for those working in a hot environment was a concern for both soldiers in Desert Storm, but also workers required to wear Personal Protective Equipment (PPE), which substantially increases their potential for heat exposure injuries.

Studies have shown side effects from PB pretreatment to be minimal for workers performing moderate exercise in hot climates. These effects include the following:

a. Slight lowering of diastolic blood pressure which is probably due to increased refractory period in cardiac conduction tissue coupled with a general dilation of blood vessels.[2]

b. Smaller pupil size which is a common effect associated with Ache inhibitors. Pupil size should not interfere with work practices with the possible exception of night time visual acuity. Recent studies involving aviation skills have shown no degradation of visual acuity or motor skills during flight operations.[8]

c. Changes in the body's thermoregulation. PB caused a slightly higher core temperature related to impaired cutaneous blood flow but increased sweating.[2]

One drawback to using PB as a pretreatment for hazardous waste and chemical demilitarization workers is the requirement to establish and maintain a sequestered blood Ache level of 20-40% prior to task performance. This is accomplished by administering 30 mg PB orally every eight hours for a period of one week prior to the start of the project.

The use of PB as a pretreatment adjunct along with postexposure atropine and oxime still does not provide any protection to the CNS.

The use of PB was adopted by the US military for use in Desert Storm (1991) but ran into roadblocks from the Food and Drug Administration and several prominent newspapers cited the military for using an "experimental" drug on military "guinea pigs".[3]

The use of PB was actually as an "Investigational New Drug" (IND) and the FDA did grant a

waiver for the use of this drug during conflicts such as Desert Storm where obtaining informed consents from all the service members involved would be impractical.[3] This presents a problem for the acceptance of any new treatment for chemical agent poisoning by the FDA because new categories for drug approval for which clear demonstration of efficacy in humans can not be shown (i.e. can't use nerve agents on humans) are not recognized by the FDA. Future approvals must be based on safety studies in humans and both safety and efficacy studies in animals which have similar physiological responses as humans.[3]

Most emergency scenarios which would warrant PB pretreatment are unplanned and require immediate actions which would preclude the use of PB as an additional safety precaution because of the time required to sequester an adequate amount of AChE.

B. Physostigmine

A relative of PB, another carbamate, Physostigmine (PHY), which is a tertiary carbamate, provides similar reversible binding with AChE but will cross the blood-brain barrier which provides a significant reduction in CNS related symptoms. Since PHY can cross the blood-brain barrier, there are potential adverse behavioral and gastrointestinal side effects related to its use as a pretreatment. Several countries have adopted PHY as a pretreatment adjunct for nerve agent poisoning.

There are also studies looking at changing the delivery systems of our pretreatment and postexposure antidotes. One change is possibly administering either PYR or PHY transdermally, which would afford a more even dosage and remove the possibility of missed doses or "double dosing", which occurred at least twice during Desert Storm. Another change would be the packaging of several drugs into one injector. A possible combination of atropine/oxime/anticonvulsant would eliminate the need for three separate injections.[6]

2. Oxime ([1-(2-(hydroximino)metyl))pyridinium-2-4(aminocarbonyl)pyridinium) dimethylether] or HI-6

HI-6 when administered with atropine has shown similar results in protective effect as the regimen of atropine and 2-Pam Chloride (standard US treatment). The drawback with using any oxime is the requirement to use a large excess of oxime to soman to rapidly reactivate AChE and provide protection, because the rate of reactivation is directly dependant on the concentration of the circulating oxime.[4]

3. Nerve Agent Scavengers.

The use of scavengers is an exciting new area of nerve agent antidote research. Current postexposure therapy and pretreatments cannot prevent occurrence of severe postexposure incapacitation, associated convulsions, and behavioral and spatial learning impairments caused by nerve agent poisoning, in particular, soman intoxication.

Research is looking into compounds that bind directly (and preferably irreversibly) with circulating nerve agent. Several human and mammalian enzymes which bind directly to nerve agents have already been identified and these include Human Butyrylcholinesterase (HuBChE), Bovine Serum AChE, and Mouse Carboxylesterase (CaE). Monoclonal antibodies against specific nerve agents (GD) are also being investigated as possible scavengers.[5,6,7]

The concept for using an agent scavenger would be to inject a sufficient quantity of the scavengers shortly before the potential exposure and the scavenger would bind directly with circulating nerve agent. These scavengers do not have a pharmacological approach to protection but act by directly binding to the circulating nerve agent before it can bind to AChE.

Initial results for HuBChE show nearly all the circulating nerve agent is sequestered (bound) within 60 seconds of administering the nerve agent and no nerve agent related CNS effects were noted. HuBChE was totally protective and the animals did not require any additional postexposure treatment. All the enzymes listed as potential scavengers have long biological half lives which would provide protection for several hours up to several days.[5]

Protection factors for these enzymes appear to be at least 4-5 X the lethal dose, which is the recommended goal for these products.[5]

Mouse Carboxylesterase (CaE) was discovered as a result of an experiment to explain the differences in protection factors between species to soman poisoning. Rodents have a much higher LD for soman than humans and other primates and researchers found these differences to be related to a rodent unique enzyme (CaE) which binds with circulating GD.[7]

Monoclonal Antibodies against soman have also been demonstrated successfully in rats. The use of a specific monoclonal antibody would alleviate concerns for immune responses against the large foreign proteins which can occur when using bovine or mouse derived enzymes as scavengers. These antibodies acted in the same fashion as the other scavengers and provided similar protection factors.[6]

Scavenger proteins may provoke immune responses in humans because of their relatively large sizes but initial studies indicate no adverse immune responses and the scavengers are readily absorbed and show at least 80% bioavailability after 90 minutes.[4-7]

One obvious advantage of using scavengers over either PB or an oxime-atropine regimen is the decreased levels of incapacitation observed in the test population (rodents). The survivors have shown less incapacitation in their motor skills, lacrimation, activity levels, etc.[4,5,7]

VI. Conclusions:

There is a definite need to reevaluate our current approach to nerve agent treatment regimens

and begin to research products which can be administered a short time before entering a toxic environment and using compounds which will minimize posttreatment incapacitation due to the binding of AChE by nerve agents.

We should also evaluate the efficacy of using scavengers to bind the agent before it complexes with AChE. These drugs appear to lessen the incapacitation suffered by the victim and may allow the worker to resume his/her activities much quicker than the current regimen.

These studies show that scavengers hold a real promise to provide military and chemical agent workers protection against nerve agent intoxication and lessen the effects of posttreatment incapacitation.

The use of novel agent scavengers may make the use of chemical weapons obsolete on the battlefield once the aggressor realizes that no additional benefit is gained by their use and the additional protection afforded the chemical or hazardous waste worker will augment our current low level agent detectors and newer protective clothing ensembles in providing the safest environment possible for our personnel. These new advances in antidotes will truly move us closer to "Prevention Rather than Treatment!!!"

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References:

1. Compton, J. A. F. : Military chemical and biological agents, 1987.

2. Cook, J.E. and Kolka, M.A.: Chronic Pyridostigmine Bromide Administration: Side Effects Among Soldiers Working in a Desert Environment, Military Medicine, 157, 5:250, 1992.

3. Berezuk, G.P., and McCarty, G.E.: Investigational Drugs and Vaccines Fielded in Support of Operation Desert Storm, Military Medicine, 157, 8:404, 1992.

4. Maxwell, D.M., Brecht, K. M., Doctor, B. P., Wolfe, A. D.: Comparison of Antidote Protection against Soman by Pyridostigmine, HI-6, and Acetylcholinesterase, The Journal of Pharmacology and Experimental Therapeutics, Vol 264, No.3, 1992.

5. Raveh, L., Grunwald, J., Marcus, D., Papier, Y., Cohen, E., and Ashani, Y.: Human Butyrylcholinesterase as a General Prophylactic Antidote for Nerve Agent Toxicity, Biochemical Pharmacology, Vol 45, No. 12, 1993.

6. Dunn, Col. M., MC, USA, and Sidell, F. R. : Progress in Medical Defense Against Nerve Agents, Jama, 1989; 262:649-652.

7. Maxwell, D.M., Brecht, K.M., Lenz, D.E., and O'Neill, B. L. : Effect of Carboxylesterase Inhibition on Carbamate Protection Against Soman Toxicity, Journal of Pharmacology and Experimental Therapeutics, Vol. 246, No. 3, 1988.

8. Wiley, R.W., Kotulak, J. C., and Behar, I.: The Effects of Pyridostigmine Bromide on Visual Performance, Aviation, Space, and Environmental Medicine, 1992.